SEQUENCE LISTING

<110> MUNROE, Donald G.
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VYAS, Tejal B.
GUPTA, Ashwani K.

<120> IDENTIFICATION OF LYSOLIPID RECEPTORS INVOLVED IN INFLAMMATORY RESPONSE

<130> 8074-8021

<140> 09/222,995
<141> 1998-12-30

<150> 60/109,885
<151> 1998-11-25

<150> 60/080,610
<151> 1998-04-03

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ggagacgacc tcccgccagg tggcctcggc attcatcgtc atcctctgtt gcgccattgt 180
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caataccttg ctctctggct ctgtcacgct gaggctgacg cctgtgcagt ggtttgcccg 360
ggacggtctg ccttcatcac gctctcggcc tctgtcttca gcctcctggc catcgccatt 420
gagcgccacg tggccattgc aaagg 445

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ggagacgacc tcccgccagg tggcctcggc cttcatcgtc atcctctgtt gcgccattgt 180
ggtggaaaac cttctggtgc tcattgcggt ggcccgaaac agcaagttcc actcggcaat 240
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ggac 364

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<212> DNA
<213> Homo sapiens

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cggagtacct gaaccccaac aaggtccagg aacactataa ttataccaag gagacgctgg 120
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ccattgtggt ggaaaacctt ctggtgctca ttgcggtggc ccgaaacagc aagttccact 240
cggcaatgta cctgtttctg ggcaacctgg ccgcctccga tctactggca ggcgtggctt 300
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tgcccggga 369

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Met Gly Ser Leu Tyr Ser

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Glu Tyr Leu Asn Pro Asn Lys Val Gln Glu His Tyr Asn Tyr Thr Lys
             10                  15                  20

gag acg ctg gaa acg cag gag acg acc tcc cgc cag gtg gcc tcg gcc   151
Glu Thr Leu Glu Thr Gln Glu Thr Thr Ser Arg Gln Val Ala Ser Ala
         25                  30                  35

ttc atc gtc atc ctc tgt tgc gcc att gtg gtg gaa aac ctt ctg gtg   199
Phe Ile Val Ile Leu Cys Cys Ala Ile Val Val Glu Asn Leu Leu Val
     40                  45                  50

ctc att gcg gtg gcc cga aac agc aag ttc cac tcg gca atg tac ctg   247
Leu Ile Ala Val Ala Arg Asn Ser Lys Phe His Ser Ala Met Tyr Leu
 55                  60                  65                  70

ttt ctg ggc aac ctg gcc gcc tcc gat cta ctg gca ggc gtg gcc ttc   295
Phe Leu Gly Asn Leu Ala Ala Ser Asp Leu Leu Ala Gly Val Ala Phe
                 75                  80                  85

gta gcc aat acc ttg ctc tct ggc tct gtc acg ctg agg ctg acg cct   343
Val Ala Asn Thr Leu Leu Ser Gly Ser Val Thr Leu Arg Leu Thr Pro
             90                  95                 100

gtg cag tgg ttt gcc cgg gag ggc tct gcc ttc atc acg ctc tcg gcc   391
Val Gln Trp Phe Ala Arg Glu Gly Ser Ala Phe Ile Thr Leu Ser Ala
        105                 110                 115

tct gtc ttc agc ctc ctg gcc atc gcc att gag cgc cac gtg gcc att   439
Ser Val Phe Ser Leu Leu Ala Ile Ala Ile Glu Arg His Val Ala Ile
    120                 125                 130

gcc aag gtc aag ctg tat ggc agc gac aag agc tgc cgc atg ctt ctg   487
Ala Lys Val Lys Leu Tyr Gly Ser Asp Lys Ser Cys Arg Met Leu Leu
135                 140                 145                 150

ctc atc ggg gcc tcg tgg ctc atc tcg ctg gtc ctc ggt ggc ctg ccc   535
Leu Ile Gly Ala Ser Trp Leu Ile Ser Leu Val Leu Gly Gly Leu Pro
                155                 160                 165

atc ctt ggc tgg aac tgc ctg ggc cac ctc gag gcc tgc tcc act gtc   583
Ile Leu Gly Trp Asn Cys Leu Gly His Leu Glu Ala Cys Ser Thr Val
            170                 175                 180

ctg cct ctc tac gcc aag cat tat gtg ctg tgc gtg gtg acc atc ttc   631
Leu Pro Leu Tyr Ala Lys His Tyr Val Leu Cys Val Val Thr Ile Phe
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185 190 195

tcc atc atc ctg ttg gcc atc gtg gcc ctg tac gtg cgc atc tac tgc 679
Ser Ile Ile Leu Leu Ala Ile Val Ala Leu Tyr Val Arg Ile Tyr Cys
200 205 210

gtg gtc cgc tca agc cac gct gac atg gcc gcc ccg cag acg cta gcc 727
Val Val Arg Ser Ser His Ala Asp Met Ala Ala Pro Gln Thr Leu Ala
215 220 225 230

ctg ctc aag acg gtc acc atc gtg cta ggc gtc ttt atc gtc tgc tgg 775
Leu Leu Lys Thr Val Thr Ile Val Leu Gly Val Phe Ile Val Cys Trp
235 240 245

ctg ccc gcc ttc agc atc ctc ctt ctg gac tat gcc tgt ccc gtc cac 823
Leu Pro Ala Phe Ser Ile Leu Leu Leu Asp Tyr Ala Cys Pro Val His
250 255 260

tcc tgc ccg atc ctc tac aaa gcc cac tac ytt ttc gcc gtc tcc acc 871
Ser Cys Pro Ile Leu Tyr Lys Ala His Tyr Xaa Phe Ala Val Ser Thr
265 270 275

ctg aat tcc ctg ctc aac ccc gtc atc tac acg tgg cgc agc cgg gac 919
Leu Asn Ser Leu Leu Asn Pro Val Ile Tyr Thr Trp Arg Ser Arg Asp
280 285 290

ctg cgg cgg gag gtg ctt cgg ccg ctg cag tgc tgg cgg ccg ggg gtg 967
Leu Arg Arg Glu Val Leu Arg Pro Leu Gln Cys Trp Arg Pro Gly Val
295 300 305 310

ggg gtg caa gga cgg agg cgg ggc ggg acc ccg ggc cac cac ctc ctg 1015
Gly Val Gln Gly Arg Arg Arg Gly Gly Thr Pro Gly His His Leu Leu
315 320 325

cca ctc cgc agc tcc agc tcc ctg gag agg ggc atg cac atg ccc acg 1063
Pro Leu Arg Ser Ser Ser Ser Leu Glu Arg Gly Met His Met Pro Thr
330 335 340

tca ccc acg ttt ctg gag ggc aac acg gtg gtc tgagggtggg ggtggaccaa 1116
Ser Pro Thr Phe Leu Glu Gly Asn Thr Val Val
345 350

caaccaggcc agggcatagg ggttcatgga aaggccactg ggtgacccca aata 1170

<210> 17
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<212> PRT

<213> Homo sapiens

<400> 17

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His Tyr Asn Tyr Thr Lys Glu Thr Leu Glu Thr Gln Glu Thr Thr Ser
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Arg Gln Val Ala Ser Ala Phe Ile Val Ile Leu Cys Cys Ala Ile Val
         35                  40                  45

Val Glu Asn Leu Leu Val Leu Ile Ala Val Ala Arg Asn Ser Lys Phe
     50                  55                  60

His Ser Ala Met Tyr Leu Phe Leu Gly Asn Leu Ala Ala Ser Asp Leu
 65                  70                  75                  80

Leu Ala Gly Val Ala Phe Val Ala Asn Thr Leu Leu Ser Gly Ser Val
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Thr Leu Arg Leu Thr Pro Val Gln Trp Phe Ala Arg Glu Gly Ser Ala
            100                 105                 110

Phe Ile Thr Leu Ser Ala Ser Val Phe Ser Leu Leu Ala Ile Ala Ile
        115                 120                 125

Glu Arg His Val Ala Ile Ala Lys Val Lys Leu Tyr Gly Ser Asp Lys
    130                 135                 140

Ser Cys Arg Met Leu Leu Leu Ile Gly Ala Ser Trp Leu Ile Ser Leu
145                 150                 155                 160

Val Leu Gly Gly Leu Pro Ile Leu Gly Trp Asn Cys Leu Gly His Leu
                165                 170                 175

Glu Ala Cys Ser Thr Val Leu Pro Leu Tyr Ala Lys His Tyr Val Leu
            180                 185                 190

Cys Val Val Thr Ile Phe Ser Ile Ile Leu Leu Ala Ile Val Ala Leu
        195                 200                 205

Tyr Val Arg Ile Tyr Cys Val Val Arg Ser Ser His Ala Asp Met Ala
    210                 215                 220

Ala Pro Gln Thr Leu Ala Leu Leu Lys Thr Val Thr Ile Val Leu Gly
225                 230                 235                 240
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Val Phe Ile Val Cys Trp Leu Pro Ala Phe Ser Ile Leu Leu Leu Asp
245 250 255

Tyr Ala Cys Pro Val His Ser Cys Pro Ile Leu Tyr Lys Ala His Tyr
260 265 270

Xaa Phe Ala Val Ser Thr Leu Asn Ser Leu Leu Asn Pro Val Ile Tyr
275 280 285

Thr Trp Arg Ser Arg Asp Leu Arg Arg Glu Val Leu Arg Pro Leu Gln
290 295 300

Cys Trp Arg Pro Gly Val Gly Val Gln Gly Arg Arg Arg Gly Gly Thr
305 310 315 320

Pro Gly His His Leu Leu Pro Leu Arg Ser Ser Ser Ser Leu Glu Arg
325 330 335

Gly Met His Met Pro Thr Ser Pro Thr Phe Leu Glu Gly Asn Thr Val
340 345 350

Val

<210> 18
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<212> DNA
<213> Homo sapiens

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ccacctttg gaagaccacg agtaacgcca ccgggctttg tcgttcaagg tgagccgtta 240
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gttatggaac gagagaccga gacagtgcga ctccgactgc ggacacgtca ccaaacgggc 360
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actcgcggtg caccggtaac ggttccagtt cgacataccg tcgctgttct cgacggcgta 480
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<210> 19
<211> 1062
<212> DNA
<213> Homo sapiens

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gtcatcctct gttgcgccat tgtggtggaa aaccttctgg tgctcattgc ggtggcccga 180
aacagcaagt tccactcggc aatgtacctg tttctgggca acctggccgc ctccgatcta 240
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<211> 1062
<212> DNA
<213> Homo sapiens

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cagtaggaga caacgcggta acaccacctt ttggaagacc acgagtaacg ccaccgggct 180
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tgcggacacg tcaccaaacg ggccctcccg agacggaagt agtgcgagag ccggagacag 360
aagtcggagg accggtagcg gtaactcgcg gtgcaccggt aacggttcca gttcgacata 420
ccgtcgctgt tctcgacggc gtacgaagac gagtagcccc ggagcaccga gtagagcgac 480
caggagccac cggacgggta ggaaccgacc ttgacggacc cggtggagct ccggacgagg 540
tgacaggacg gagagatgcg gttcgtaata cacgacacgc accactggta gaagaggtag 600

taggacaacc ggcagcaccg ggacatgcac gcgtagatga cgcaccaggc gagttcggtg 660
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cagaaatagc agacgaccga cgggcggaag tcgtaggagg aagacctgat acggacaggg 780
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<211> 352
<212> PRT
<213> Rattus sp.

<400> 21
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Arg Lys Val Ala Ser Ala Phe Ile Ile Ile Leu Cys Cys Ala Ile Val
35 40 45

Val Glu Asn Leu Leu Val Leu Ile Ala Val Ala Arg Asn Ser Lys Phe
50 55 60

His Ser Ala Met Tyr Leu Phe Leu Gly Asn Leu Ala Ala Ser Asp Leu
65 70 75 80

Leu Ala Gly Val Ala Phe Val Ala Asn Thr Leu Leu Ser Gly Pro Val
85 90 95

Thr Leu Ser Leu Thr Pro Leu Gln Trp Phe Ala Arg Glu Gly Ser Ala
100 105 110

Phe Ile Thr Leu Ser Ala Ser Val Phe Ser Leu Leu Ala Ile Ala Ile
115 120 125

Glu Arg Gln Val Ala Ile Ala Lys Val Lys Leu Tyr Gly Ser Asp Lys
130 135 140

Ser Cys Arg Met Leu Met Leu Ile Gly Ala Ser Trp Leu Ile Ser Leu
145 150 155 160

Ile Leu Gly Gly Leu Pro Ile Leu Gly Trp Asn Cys Leu Asp His Leu
165 170 175

Glu Ala Cys Ser Thr Val Leu Pro Leu Tyr Ala Lys His Tyr Val Leu
180 185 190

Cys Val Val Thr Ile Phe Ser Val Ile Leu Leu Ala Ile Val Ala Leu
195 200 205

Tyr Val Arg Ile Tyr Phe Val Val Arg Ser Ser His Ala Asp Val Ala
210 215 220

Gly Pro Gln Thr Leu Ala Leu Leu Lys Thr Val Thr Ile Val Leu Gly
225 230 235 240

Val Phe Ile Ile Cys Trp Leu Pro Ala Phe Ser Ile Leu Leu Leu Asp
245 250 255

Ser Thr Cys Pro Val Arg Ala Cys Pro Val Leu Tyr Lys Ala His Tyr
260 265 270

Phe Phe Ala Phe Ala Thr Leu Asn Ser Leu Leu Asn Pro Val Ile Tyr
275 280 285

Thr Trp Arg Ser Arg Asp Leu Arg Arg Glu Val Leu Arg Pro Leu Leu
290 295 300

Cys Trp Arg Gln Gly Lys Gly Ala Thr Gly Arg Arg Gly Gly Asn Pro
305 310 315 320

Gly His Arg Leu Leu Pro Leu Arg Ser Ser Ser Ser Leu Glu Arg Gly
325 330 335

Leu His Met Pro Thr Ser Pro Thr Phe Leu Glu Gly Asn Thr Val Val
340 345 350

<210> 22
<211> 353
<212> PRT
<213> Homo sapiens

<400> 22
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His Tyr Asn Tyr Thr Lys Glu Thr Leu Glu Thr Gln Glu Thr Thr Ser

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35 40 45

Val Glu Asn Leu Leu Val Leu Ile Ala Val Ala Arg Asn Ser Lys Phe
50 55 60

His Ser Ala Met Tyr Leu Phe Leu Gly Asn Leu Ala Ala Ser Asp Leu
65 70 75 80

Leu Ala Gly Val Ala Phe Val Ala Asn Thr Leu Leu Ser Gly Ser Val
85 90 95

Thr Leu Arg Leu Thr Pro Val Gln Trp Phe Ala Arg Glu Gly Ser Ala
100 105 110

Phe Ile Thr Leu Ser Ala Ser Val Phe Ser Leu Leu Ala Ile Ala Ile
115 120 125

Glu Arg His Val Ala Ile Ala Lys Val Lys Leu Tyr Gly Ser Asp Lys
130 135 140

Ser Cys Arg Met Leu Leu Leu Ile Gly Ala Ser Trp Leu Ile Ser Leu
145 150 155 160

Val Leu Gly Gly Leu Pro Ile Leu Gly Trp Asn Cys Leu Gly His Leu
165 170 175

Glu Ala Cys Ser Thr Val Leu Pro Leu Tyr Ala Lys His Tyr Val Leu
180 185 190

Cys Val Val Thr Ile Phe Ser Ile Ile Leu Leu Ala Val Val Ala Leu
195 200 205

Tyr Val Arg Ile Tyr Cys Val Val Arg Ser Ser His Ala Asp Met Ala
210 215 220

Ala Pro Gln Thr Leu Ala Leu Leu Lys Thr Val Thr Ile Val Leu Gly
225 230 235 240

Val Phe Ile Val Cys Trp Leu Pro Ala Phe Ser Ile Leu Leu Leu Asp
245 250 255

Tyr Ala Cys Pro Val His Ser Cys Pro Ile Leu Tyr Lys Ala His Tyr
260 265 270

Leu Phe Ala Val Ser Thr Leu Asn Ser Leu Leu Asn Pro Val Ile Tyr

275 280 285

Thr Trp Arg Ser Arg Asp Leu Arg Arg Glu Val Leu Arg Pro Leu Gln
290 295 300

Cys Trp Arg Pro Gly Val Gly Val Gln Gly Arg Arg Arg Gly Gly Thr
305 310 315 320

Pro Gly His His Leu Leu Pro Leu Arg Ser Ser Ser Ser Leu Glu Arg
325 330 335

Gly Met His Met Pro Thr Ser Pro Thr Phe Leu Glu Gly Asn Thr Val
340 345 350

Val

<210> 23
<211> 351
<212> PRT
<213> Homo sapiens

<400> 23
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1 5 10 15

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20 25 30

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35 40 45

Thr Asn Leu Leu Val Ile Ala Ala Ile Ala Ser Asn Arg Arg Phe His
50 55 60

Gln Pro Ile Tyr Tyr Leu Leu Gly Asn Leu Ala Ala Ala Asp Leu Phe
65 70 75 80

Ala Gly Val Ala Tyr Leu Phe Leu Met Phe His Thr Gly Pro Arg Thr
85 90 95

Ala Arg Leu Ser Leu Glu Gly Trp Phe Leu Arg Gln Gly Leu Leu Asp
100 105 110

Thr Ser Leu Thr Ala Ser Val Ala Thr Leu Leu Ala Ile Ala Val Glu
115 120 125

Arg His Arg Ser Val Met Ala Val Gln Leu His Ser Arg Leu Pro Arg
130 135 140

Gly Arg Val Val Met Leu Ile Val Gly Val Trp Val Ala Ala Leu Gly
145 150 155 160

Leu Gly Leu Leu Pro Ala His Ser Trp His Cys Leu Cys Ala Leu Asp
165 170 175

Arg Cys Ser Arg Met Ala Pro Leu Leu Ser Arg Ser Tyr Leu Ala Val
180 185 190

Trp Ala Leu Ser Ser Leu Leu Val Phe Leu Leu Met Val Ala Val Tyr
195 200 205

Thr Arg Ile Phe Phe Tyr Val Arg Arg Arg Val Gln Arg Met Ala Glu
210 215 220

His Val Ser Cys His Pro Arg Tyr Arg Glu Thr Thr Leu Ser Leu Val
225 230 235 240

Lys Thr Val Val Ile Ile Leu Gly Ala Phe Val Val Cys Trp Thr Pro
245 250 255

Gly Gln Val Val Leu Leu Leu Asp Gly Leu Gly Cys Glu Ser Cys Asn
260 265 270

Val Leu Ala Val Glu Lys Tyr Phe Leu Leu Leu Ala Glu Ala Asn Ser
275 280 285

Leu Val Asn Ala Ala Val Tyr Ser Cys Arg Asp Ala Glu Met Arg Arg
290 295 300

Thr Phe Arg Arg Leu Leu Cys Cys Ala Cys Leu Arg Gln Ser Thr Arg
305 310 315 320

Glu Ser Val His Tyr Thr Ser Ser Ala Gln Gly Gly Ala Ser Thr Arg
325 330 335

Ile Met Leu Pro Glu Asn Gly His Pro Leu Met Asp Ser Thr Leu
340 345 350

<210> 24
<211> 1056
<212> DNA
<213> Homo sapiens

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13B). Therefore, induction of inflammatory gene expression pathways is a conserved feature of EDG-4 in humans and rats, and likely reflects a fundamental biological aspect of receptor function.

Together, these results suggest that the SRE response is a shared feature of many different edg/lysolipid receptors, and can be used to verify the response of intact, functional receptors to their cognate agonist(s). On the other hand, the NF-κB response is shared by a subset of edg/lysolipid receptors which are specialized to mobilize inflammatory gene expression and immune system recruitment. Since EDG-1, EDG-3, EDG-4 and EDG-7 are all S1P/SPC receptors, their varying and even overlapping tissue distribution and inducibility frustrate the meaningful design, screening and therapeutic testing of anti-inflammatory S1P analogs unless the subtype specificity of inflammatory signaling is appreciated. This complexity highlights the value and utility of the recombinant inflammatory lysolipid receptors and the functional assays specified herein.

EXAMPLE 12. Identification of human expressed sequence tags (ESTs) homologous to rat H218 (EDG-4).

A BLAST search of the complete GenBank database was conducted with the sequence of an oligonucleotide RE4_181F [3'-GAGAAGGTTCAGGAACACTACAATTACACCAA GGA-3'] (SEQ ID NO:1), based on the sequence of rat EDG-4. The search identified a human EST (GenBank accession AA804628), which was 88% identical to the corresponding region of rat EDG-4 cDNA (GenBank accession U10699). A subsequent TBLASTN search of the EST database using the predicted polypeptide product of the rat EDG-4 cDNA (according to accession number U10699) revealed 2 other matching EST's (accession AA827835 and AA834537) in addition to the original human EST. The 3 EST's encompassed the predicted translation start site of human EDG-4 (based on similarity to rat EDG-4), overlapped each other extensively, and together spanned some 109 codons of the N-terminal portion of the human EDG-4 polypeptide (Figure 14). The predicted fragment of the human EDG-4 polypeptide showed 90.1% identity and 93.3% similarity to the equivalent fragment of rat EDG-4, suggesting the human polypeptide is an ortholog of the rat EDG-4 gene product, rather than a closely related gene product. A BLAST search was then conducted with the complete sequence of rat EDG-4 cDNA (accession number U10699) against the EST database. In addition to the previously identified EST's, 2 EST's apparently derived from the 3'-untranslated region of human EDG-4 cDNA adjacent to the

poly(A) tail were found (AA767046 and N93714). Of the 5 human EST's identified in total, only N93714 was present in the public database before February 19, 1998. This EST was derived from the 3' end of a 1421 bp cDNA insert which contained no coding region. The closest match recorded in the DBEST database entry (accession 500502) was a cGMP phosphodiesterase. The 5' end of the clone had been sequenced and given the GenBank accession W21101; however, similarity to other cDNAs was obscured by the presence of an Alu sequence.

EXAMPLE 13. Survey of potential cDNA sources using 5' end and 3' end diagnostic PCR.

To evaluate possible sources of human EDG-4 cDNA from HeLa cells (which express the inflammatory S1P/SPC receptor) and lung (a predominant site of EDG-4 expression in rat) for the presence of the desired cDNA fragments, diagnostic PCR primers were designed from the cluster of 5' end EST's (AA804628, AA834537 and AA827835) and 3' end EST's (N93714 and AA767046):

5' end primers:

HE4-DF1 [5'-ATTATACCAAGGAGACGCTGGAAAC-3'] (SEQ ID NO:2)
HE4-DR1 [5'-AGAGAGCAAGGTATTGGCTACGAAG-3'] (SEQ ID NO:3)

3' end primers:

HE4-DF2 [5'-TCCTCTCCTCGTCACATTTCCC-3'] (SEQ ID NO.4)
HE4-DR2 [5'-GCATTCACAAGAAATTACTCTGAGGC-3'] (SEQ ID NO:5)

Template sources: 1) cDNA library from WI-38 lung fibroblasts (Origene Technologies Inc., Cat. DLH-102); 2) cDNA library from human lung (Clontech, Cat. 7114-1); 3) cDNA library from HeLa cells (Invitrogen, Cat. A550-26); 4) First strand cDNA prepared in-house from HeLa cell total RNA. Each template was amplified with each pair of primers using the Expand™ PCR system from Boehringer Mannheim (Cat.1681-842) .

Each reaction contained the following reagents:

2 µl	10x PCR Buffer 3
0.4 µl	25mM dNTP mix
0.6 µl	Primer HE4-DF1 or HE4-DF2 (10 µM)
0.6 µl	Primer HE4-DR1 or HE4-DR2 (10 µM)

amplified, containing the complete coding region. These primers were used in a PCR reaction with the WI-38 human lung fibroblast cDNA library (Origene) as follows:

HE4-DF3 [5'-GAGCCCCACCATGGGCAGCTTGTACT-3'] (SEQ ID NO.6)
HE4-DR2 [5'-GCATTCACAAGAAATTACTCTGAGGC-3'] (SEQ ID NO.7)

Each reaction contained the following reagents:

5 µl	10x PCR Buffer 3
1.0 µl	25mM dNTP mix
1.5 µl	Primer HE4-DF3 (10 µM)
1.5 µl	Primer HE4-DR2 (10 µM)
0.75 µl	Expand™ enzyme (2 units)
39.25 µl	water
1 µl	cDNA template (250 ng or 500 ng of DNA)

PCR conditions:

Incubate:	94°C for 2 min
10 cycles:	94°C for 40 sec
	60°C for 40 sec
	68°C for 5 min
25 cycles:	94°C for 40 sec
	60°C for 40 sec
	68°C for 3 min
Incubate:	68°C for 8 min
Hold:	4°C

Amplified reactions from 250 ng (tube 227-45) and 500 ng (227-50) of cDNA template each contained 3 PCR products 2 kb or larger. The PCR reaction and the DNA fragments from the gel were purified using QIAquick PCR purification kit (Qiagen Cat. 28106) and QIAquick gel extraction kit (Qiagen, Cat. 28704), respectively. Diagnostic PCR reactions were carried out on each of the 3 PCR products, and all

3 yielded the expected diagnostic PCR products using both the 5' end and 3' end primer pairs. Because they differed in size (~2 kb, 2.2 and 2.4 kb) and yet amplified with primers from the translation start and the 3'-untranslated region, all 3 may represent different alternatively spliced edg-4 transcripts.

The 3 PCR products were used as templates to reamplify human edg-4 with primers containing restriction sites suitable for cloning into an expression vector. Two different 3'-end primers were selected with longer (HE4-DR3) or shorter (HE4-DR4) 3'-untranslated regions. The following PCR primers and PCR conditions were used:

HE4-DF4 [5'-TTTAAAAAGCTTCCCACCATGGGCAGCTTGTACT-3'] (SEQ ID NO: 8)
HE4-DR3 [5'-TATATATCTAGACATTCACAAGAAATTACTCTGAGGC-3'] (SEQ ID NO: 9)
HE4-DR4 [5'-TATATATCTAGAGGAAATGTGACGAGGAGAGG-3'] (SEQ ID NO: 10)

Each reaction contained the following reagents:

5 µl	10x PCR Buffer 3
1.0 µl	25mM dNTP mix
1.5 µl	Primer HE4-DF4 (10 µM)
1.5 µl	Primer HE4-DR3 or HE4-DR4 (10 µM)
0.75 µl	Expand ™ enzyme (5 units)
39.25 µl	water
1 µl	DNA

PCR conditions:

Incubate:	94°C for 2 min
28 cycles:	94°C for 40 sec
	60°C for 40 sec
	68°C for 3.5 min
Incubate:	68°C for 8 min
Hold:	4°C

A pair of primers was designed from two ends of reading frame of human edg-4 cDNA sequence to engineer the edg-4 open reading frame into a vector designed for GFP fusion protein expression, with the GFP tag carboxy-terminal to the full-length EDG-4 polypeptide:

5'-End Primer: Contains Site for Kpn I enzyme, and optimized (Kozak) translation initiation sequence:

HE4-ATG KpnF: [5'-TTTAAAGGTACCGCCACCATGGGCAGCTTGTAC-3'] (SEQ ID NO:11)

3'-End Primer: Contains site for XbaI enzyme, and lacks naturally-occurring edg-4 stop codon:

HE4-xba/1096R: [5'-TATATATCTAGAGACCACCGTGTTGCCCTCCAG-3'] (SEQ ID NO:12)

pc3-hedg4#36 plasmid DNA was amplified with the above pair of primers under the following conditions of PCR amplification, using the Expand™ PCR system from Boehringer Mannheim (Cat. 1681-842).

The reaction contained the following reagents:

5 µl of 10x PCR Buffer 3
1.0 µl of 25mM dNTP mix
1.5 µl of Primer HE4-ATG KpnF (10 pmol/l)
1.5 µl of Primer HE4-xba/1096R (10pmol/l)
0.75 µl of Enzyme (2 units)
39.25 µl water
1 µl DNA

PCR conditions:

Incubate:	94°C for 2 min
10 cycles:	94°C for 1 min
	50°C for 1 min